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Anærobic Infections in Animals.

Discussion on Professor Gaiger's Paper.

Mr. Dalling's Views.

Prior to the discussion on his paper, Professor Gaiger exhibited the series of lantern slides set out below. They were excellently reproduced and were followed closely and with great interest. The list of slides is as follows :—

1. *Vibrion septique* on the peritoneum showing filament formation, in culture, and shewing flagella.
2. *Bacillus welchii* in culture.
3. *Bacillus chauvæi* in culture, and in a muscle smear.
4. *Bacillus ædematiens* in a muscle smear and on the serous membranes of a guinea-pig.
5. *Bacillus histolyticus* in culture, and in a muscle smear from a guinea-pig.
6. *Bacillus tetani* from a wound scraping.
7. Surface culture of *Vibrion septique* on serum agar.
8. Surface culture of *Bacillus welchii* on blood agar.
9. Deep culture of *Vibrion septique* in glucose agar.
10. Deep culture of *Bacillus welchii* in glucose agar.
11. Deep culture of *B. histolyticus* in glucose agar.
12. Deep culture of *B. sporogenes* in glucose agar.
13. Horse with *Vibrion septique* infection.
14. Horse with *B. histolyticus* and *B. ædematiens* infection.
15. Braxy stomach. — Shewing the area of inflammation constantly present, being the place of penetration of the *Vibrion septique*.
16. Braxy stomach section ×30.
17. Braxy stomach, section ×50. — Shewing engorgement of the blood vessels of the mucosa, necrosis of the surface of the mucosa, migration of leucocytes, sub-mucous connective tissue showing indistinct staining.
18. Braxy stomach, section ×30. — Shewing engorged mucosa vessels, a heavy zone of migrated leucocytes, necrosis of the mucosa superficially, and the sub-mucous connective tissue indistinct. The zone of leucocytes does not border an infarct.

19. Normal sheeps' stomach section. — Shewing the differences from the last three.
20. Braxy stomach section. — Shewing the *Vibrion septique* in a small cluster surrounded by migrated leucocytes.
21. Braxy stomach section. — Shewing the same under a higher power.
22. Braxy stomach section. — Under a high power to show the bacillus sporulating in the stomach wall.
23. Braxy stomach section. — Shewing that where large numbers of the bacilli are present the leucocytes have been overcome, and are few in numbers. The masses of bacilli have a felt-like appearance.
24. Same under a higher power.
25. Braxy bacillus commencing to form filaments on the peritoneum of a guinea-pig.
26. Braxy bacillus in diagram to show the arrangement of flagella.
27. The flagella of *B. typhosus* for comparison shewing the tresses of flagella, such as are not seen in the braxy bacillus.
28. Braxy stomach section. — Shewing a place where severe inflammation has resulted in hæmorrhage into the mucosa and submucosa. This slide shows that nothing resembling an infarct is produced by a hæmorrhage, nor are leucocytes seen to be attacking the hæmorrhagic area as has been erroneously suggested.
29. Braxy stomach section. — Shewing a hæmorrhagic area under a high power to show how the masses of red blood corpuscles do not kill the tissue cells of the stomach wall to form an infarct. The nuclei of the stomach wall tissue are seen taking the stain normally.
30. Lamb Dysentery. — Ulcers in the small intestine.
31. Lamb Dysentery. — Ulcer under a low power of the microscope. Shewing necrosis.

32. Lamb Dysentery. — Ulcer tissue under a high-power shewing the *B.welchii*, one of the secondary organisms of the disease.

33. Lamb Dysentery. — Masses of intestines adherent to each other and to the wall of the abdomen.

34. Table shewing anti-*Bacillus chauvæi* serum does not protect guinea-pigs against the *Vibrion septique*.

Mr. T. Dalling opened the discussion on Professor Gaiger's paper with a thoroughly comprehensive address, which was also illustrated by excellent lantern slides. He said :—

When Mr. Gaiger invited me to open the discussion on his paper, I gladly accepted the invitation, because I felt sure that he would bring forward material of the greatest interest to all branches of the profession and that he would lay before us some of his own findings in his researches into animal diseases. I felt also, having been associated with him for nearly four years in much of his work on anærobes, that his writings would prove abundantly full of useful knowledge, both to the practitioner and to men who, like myself, are engaged in the laboratory aspect of disease questions. My expectations have been fully realised. In bringing forward this paper, Mr. Gaiger has done a very great service to the Veterinary Profession in that he has condensed for us into a work of readable size, practically all that has been written by workers in all parts of the world on the part played by anærobes in the causation of animal diseases. There is nothing more fatiguing than the hunting up of articles one has read on some special subject, and one can readily realise the immense task Mr. Gaiger imposed on himself when he set out to study the various articles on anærobes found in practically all medical and veterinary scientific journals and to condense them into such a small compact space, keeping the essentials always in the foreground. And not only has he given us the findings of others, he has included much of his own work, some of which has been subjected to the severest of criticism in the past, but which, by his results in the field, has been clearly shown to have been in the right direction. Again he supplies us with an excellent list of references at the end of his paper, so

that we can at a glance find the various articles on his subject should we wish to consult them in detail: for this alone we are deeply indebted to him, for all laboratory workers are keen to have a full list of references to articles on the problems upon which they are engaged.

The subject of anærobic infections of animals is one brimful of points for discussion: I should like to limit my remarks to three items, all referred to in this paper. They are:—

(1) Organisms associated with Blackquarter and immunisation against them.
(2) The association of *B. welchii* with animal diseases, with special reference to lamb dysentery.
(3) Some general debatable points.

Organisms associated with Blackquarter, and Immunisation against them.

That Blackquarter is the result of the action of more than one organism is becoming more apparent as time progresses and as the results of various bits of research come to light. Undoubtedly the similarity between *B. chauvœi* and the *Vibrion septique* has led to much confusion in the identity of the organism found in the muscle lesion, but now that we have a clear conception of the differences between the two, and now that we understand what a pure culture of an anærobe really is, it becomes more and more apparent that *Vibrion septique* has been labelled *B. chauvœi* on many occasions or that so-called pure cultures of *B. chauvœi* were really mixtures of that organism and others.

It has been our privilege to examine some strains of so-called *B. chauvœi* isolated from Rauschbrand (Blackquarter) cases by Continental workers and from which agents used in the immunisation against that disease have been made and used. Of eighteen such cultures examined, only two have been identified as *B. chauvœi*: fifteen were pure cultures of *Vibrion septique* and one was a pure culture of *B. histolyticus*. It will thus be evident that, on the Continent, at least, organisms other than *B. chauvœi*, notably the *Vibrion septique*, are found in pure culture in association with Blackquarter lesions.

From American laboratories we have received three cultures of *B. chauvœi*, all of which we have been able to confirm as pure cultures of that organism.

It may be of interest here to state briefly our procedure in the identifying of anærobic cultures. When a culture is received or isolated, it is examined microscopically for evidence of obvious contamination. If the organism be a sporing one, sporulation is encouraged by growth on meat containing media, and capillary tubes filled with the sporulating medium are heated at 80°C. for twenty minutes in an endeavour to kill off all vegetative forms, leaving the spores viable for further work. Cultures made from the heated material are used for colony production work, both on the surface and in the depth of solid media. Personally, I greatly prefer the surface colony method, using blood agar as a medium and a direct stream of hydrogen as a means to create efficient anærobiosis. Individual colonies are picked off and cultures from them are subjected to further plating after growth in different media, *e.g.*, milk, alkaline, egg, etc. This plating is continued till we are reasonably sure that a pure culture is present. Sugar fermentation tests are now carried out and noted. Guinea-pigs are inoculated intramuscularly with the now so-called " pure " culture and the post-mortem effects studied. Cultures are filtered and the toxicity in guinea-pigs and mice observed. Lastly, guinea-pigs are protected with the various anærobe anti-sera, and several M.L.D.'s. of the culture are inoculated. By such means one is fairly safe in concluding which anærobe one is dealing with and whether one or more types are present.

Mr. Gaiger has pointed out that the lesions produced in sheep by artificial inoculation with cultures of *B. chauvœi* and *Vibrion septique* are identical. I agree with this, and it would not be unreasonable to expect to find *Vibrion septique* as a cause of Blackquarter in cattle and sheep in this country. We have received samples of muscle taken from cattle dead of Blackquarter from eight different districts in Britain and have been able in each case to isolate *B. chauvœi*, but never *Vibrion septique*. However, eight is a small number from which to make any deductions, and we live in the hope of receiving many more samples from veterinary practitioners, from which we can examine and draw conclusions. Bosworth and Allen state that in one case out of six they have

been able to demonstrate the presence of *Vibrion septique* in muscle from Blackquarter.

There always exists an element of doubt in my mind as to the part played by *Vibrion septique* isolated along with other ærobes and anærobes from any tissue which may have been in contact with the soil, or from an animal which has lain dead for some time before the tissue was collected. One cannot lose sight of the fact that *Vibrion septique*, as Mr. Gaiger and others have found, is present in the soil in most parts, is a constant inhabitant of the alimentary tract of most herbivora, and penetrates into the tissues of the animal with other organisms soon after death: thus, the isolating of *Vibrion septique* from the tissues of a dead animal or from a tissue (*e.g.*, muscle) that has come in contact with soil is of little consequence. It will require careful work still before we can state definitely that *Vibrion septique* is the cause of Blackquarter in a proportion of the cases found in cattle and sheep in this country. From Continental work, however, we must regard it as a likely cause, and hence, in immunising animals against the disease, immunity should be produced against both organisms.

Immunisation.

Considerable attention has been given to the use of filtrates of anærobes in the production of immunity against disease and during the last year or so many experiments have been carried out at the Wellcome Physiological Research Laboratories on the subject. Bosworth and Allen have just published the results of some of this work, with the latter part of which I was associated. It may interest you to hear of some of this work.

Protection against B. chauvœi.

We have not been able to obtain a filtrate from *B. chauvœi* toxic for mice or guinea-pigs. Mice will withstand up to 1 c.c. of any of our filtrates intravenously, and no dose we have given to guinea-pigs has ever had the slightest toxic effect.

Many experiments have been carried out to show the period of growth required for the production of the best antigenic filtrate. It has been agreed that the filtrate from a four-day growth gives best results.

Protective Value of Filtrate in Guinea-pigs.

Table 1 shows that a single inoculation of 2·5 c.c. filtrate subcutaneously protected against one certain M.L.D. culture in 80 per cent. of cases, the time between the injections of the filtrate and culture being one month.

TABLE I.

Guinea-pig.	Filtrate.	Culture. (One month later).	Result.
A	2·5 c.c.	0·5 c.c. (One M.L.D.).	Lived.
B	,,	,,	Lived.
C	,,	,,	Lived.
D	,,	,,	Dead 48 hours.
E	,,	,,	Lived.
F	,,	,,	Lived.
G	,,	,,	Lived.
H	,,	,,	Dead 48 hours.
J.	,,	,,	Lived.
K	Nil	,,	Dead overnight.
L	Nil	,,	Dead overnight.
M	Nil	,,	Dead overnight.
N	Nil	,,	Dead overnight.

A higher degree of immunity has been produced by giving two inoculations at a month's interval, when 100 per cent. protection was got. This is shown in Table II.

TABLE II.

Guinea-pig.	1st inoc.	2nd inoc. (1 month later).	Culture. (14 days later).	Results.
A	2 c.c. filtrate	2 c.c. filtrate	0.5 c.c. (1 M.L.D.)	Lived
B	,,	,,	,,	Lived.
C	,,	,,	,,	Lived.
D	,,	,,	,,	Lived.
E	Nil	Nil	,,	Dead o.n.
F	Nil	Nil	,,	Dead o.n.
G	Nil	Nil	,,	Dead o.n.
H	Nil	Nil	,,	Dead o.n.

Protective Value of Filtrate in Sheep.

Four sheep received two inoculations of 5 c.c. *B. chauvæi* filtrate at a fortnight's interval. Each received 3 c.c. culture *B. chauvæi* at varying times up to seven months after the second inoculation; all survived the test, while two sheep which had received

no protection died in twenty-four to thirty hours after receiving the same dose of culture.

Thus it can be confidently stated that the filtrate of *B. chauvœi* has a marked antigenic value.

Protection against Vibrion septique.

Vibrion septique in young cultures produces a highly potent toxin. The toxicity can readily be shown by injecting the filtrate intravenously into mice or guinea-pigs. Table III. shows the results in mice; Table IV. the results in guinea-pigs.

Table III.

Mouse.	Dose of Toxin.	Results.
A	0·25 c.c.	+ 3 mins.
B	0·25 c.c.	+ 2 mins.
C	0·1 c.c.	+ 5 mins.
D	0·1 c.c.	+ 4 mins.
E	0·05 c.c.	+ 55 mins.
F	0·05 c.c.	+ 55 mins.
G	0.025 c.c.	+130 mins.
H	0.025 c.c.	+114 mins.
J	0·01 c.c.	+ 48 hrs. approx.
K	0·01 c.c.	+ 24 hrs. approx.
L	0·005 c.c.	+ 72 hrs. approx.
M	0·005 c.c.	+ 72 hrs. approx.
N	0·0025 c.c.	—
O	0·0025 c.c.	—
P	0·001 c.c.	—
Q	0·001 c.c.	—

\+ = died. — = survived.

Table IV.

Guinea-pigs.	Dose of Toxin.	Results.
A	0·25 c.c.	+ 6 mins.
B	0·2 c.c.	+ 7 mins.
C	0·15 c.c.	+ 63 mins.
D	0·1 c.c.	+ 21mins.
E	0.09 c.c.	+ 82 mins.
F	0.08 c.c.	+145 mins.
G	0.07 c.c.	+150 mins.
H	0·06 c.c.	+ 5 hrs.
J	0·05 c.c.	+ o.n.
K	0·04 c.c.	—
L	0·03 c.c.	—

It was found that the use of a pure toxic filtrate produced necrotic lesions in guinea-pigs and marked local œdema in sheep on inoculation, hence a mixture of toxin and antitoxin was used for immunising purposes—a 25 per cent. under neutralised toxin giving good results. Table V. shows how such neutralisation is carried out. The smallest dose of antitoxin that will protect against a certain lethal dose of the toxin is taken as the neutralising dose of toxin. By calculation the amount of antitoxin required to neutralise the amount in a given batch of toxin is ascertained. 25 per cent. of this amount is added to the batch.

TABLE V.

Mouse.	Toxin.	Anti-toxin.	Result.
A	·5 c.c.	——	+ 3 mins.
B	·5 c.c.	——	+ 5 mins.
C	·25 c.c.	——	+ 5 mins.
D	·25 c.c.	——	+ 7 mins.
E	·25 c.c.	·003 c.c.	+ 3 mins.
F	·5 c.c.	·006 c.c.	+ 6 mins.
G	·5 c.c.	·012 c.c.	+ o.n.
H	·5 c.c.	·025 c.c.	—
J	·5 c.c.	·05 c.c.	—
K	·5 c.c.	1 c.c.	—

It has also been ascertained that the filtrate from a forty-eight hour culture is of good antigenic value.

Protective Value of Mixture in Guinea-pigs.

Table VI. shows that one inoculation of a *Vibrion septique* mixture produces a certain degree of immunity only, while Table VII. shows that a double inoculation adds greatly to the amount of immunity produced.

TABLE VI.

Guinea-pig.	V.S. Mixture.	V.S. Toxin (Intravenously).	Result.
A	2 c.c.	·25 c.c.	Lived.
B	2 c.c.	·25 c.c.	Lived.
C	2 c.c.	·25 c.c.	Died 48 hrs.
D	2 c.c.	·25 c.c.	Died 1 hr.
E	——	·1 c.c.	Died 5 hrs.
F	——	·1 c.c.	Died 13 mins.

Toxin injected a fortnight after mixture.

TABLE VII.

Guinea-pig.	1st inoc.	2nd inoc.	Culture.	Result.
A	2 c.c.	2 c.c.	·05 c.c.	Lived
B	2 c.c.	2 c.c.	·05 c.c.	Lived.
C	2 c.c.	2 c.c.	·05 c.c.	Lived.
D	2 c.c.	2 c.c.	·05 c.c.	Lived.
E	2 c.c.	2 c.c.	·05 c.c.	Lived.
F	——	——	·05 c.c.	Dead 24 hrs.
G	——	——	·05 c.c.	Dead 24 hrs.

Protective Value of Filtrate in Sheep.

It has been shown conclusively that one dose of mixture fails to protect sheep against a lethal dose of *Vibrion septique* culture, but that a double inoculation given at intervals up to two months leaves the animals immune when tested up to six months after the second inoculation.

Immunity to B. chauvœi and Vibrion septique in the same animal.

It has been shown that by a simultaneous inoculation of *B. chauvœi* filtrate and *Vibrion septique* toxin (antitoxin mixture) guinea-pigs and sheep are rendered immune to inoculations of both organisms. The following is a summary of one experiment. Sheep A, B, C, D, and E all received a subcutaneous inoculation of 5 c.c. *B. chauvœi* filtrate and 5 c.c. 25 per cent. underneutralised *Vibrion septique* filtrate.

Two months later the dose was repeated. A and B received 3 c.c. *B. chauvœi* culture one month after the second inoculation and 4 c.c. *Vibrion septique* three weeks later. All survived. C, D, and E received 4 c.c. *Vibrion septique* three months after the second inoculation, and 3 c.c. *B. chauvœi* five months later. All survived. With each cultural test, control sheep were used, and all died in twenty-four hours to thirty-six hours. Thus it is very obvious that sheep at any rate can be immunised against both *B. chauvœi* and *Vibrion septique*, and, in the light of our present day knowledge, any preparation used for the protection of animals against Blackquarter should be one that will immunise against both organisms. Filtrates of the one and an underneutralised mixture of the other appear to give rise to a high degree of protection.

The Association of B. welchii with Animal Diseases with special reference to Lamb Dysentery.

Whether *B. welchii* actually causes disease in animals has never been ascertained satisfactorily. That it is found present in the tissues of diseased animals is well known, and Mr. Gaiger enumerates examples of where various workers have found it, including results of work done in the A.D.R.A. laboratory. Nakamura, in the *Japanese Journal of Veterinary Science*, June, 1922, records an outbreak of diarrhœa in young chicks with fatal results, from cases of which he isolated an organism closely resembling *B. welchii*, except in its fermentation action on mannite, and with cultures of which he was able to reproduce the disease. Personally, I have isolated *B. welchii* from the brain substance of sheep killed while suffering from trembling, as quoted by Mr. Gaiger, and since then have isolated it from the brain substance of one dog killed while affected with chorea following distemper, and from the same tissue of one sheep destroyed while affected with a disease presenting acute nervous symptoms—a disease peculiar to ewes to the Herdwick breed and affecting them at or immediately after lambing. Further, a culture taken from a human source came into my hands the other day, having been isolated from the blood during life. It remains for further research to show the true part played by this organism in animal diseases, but one cannot but hold that a pathogenic anærobe found in nervous structures in the course of disease presenting nervous symptoms plays more than a secondary part. These organisms I have isolated from the brain substances of these various animals are highly pathogenic, when cultured and inoculated into guinea-pigs. I have no proof to offer to show that the organism plays more than a very secondary part, but one cannot but feel that research may ultimately show that some considerable importance must be attached to its presence there.

I agree that spores of *B. welchii* are difficult to find in artificial culture, and though I have tried to encourage sporulation on all the various mediums recognised as good for that purpose, I do not think I have seen more than six spores during all my work. It is evident, however, that cultures of *B. welchii*,

grown on a medium containing meat, become resistant to heat, for in attempting to isolate cultures from a mixture I have been able to heat such meat cultures for fifteen minutes at 80° C., and recover cultures of *B. welchii*. Spores may be demonstrated by the incubation of the whole carcase of a guinea-pig dead from a *B. welchii* infection.

The organism produces a fairly potent toxin in young cultures. I usually work with a culture grown 12—15 hours at 37° C., for the production of the best toxin. In my experience the toxin is not stable, and if kept even in an ice room it is quickly reduced in potency. To test the toxicity of *B. welchii* filtrate we use mice, inoculating them intravenously. An example of such a test is given in Table VIII.

Table VIII.

Mouse.	Toxin.	Result.
1	0·25 c.c.	+ overnight.
2	0·25 c.c.	+ overnight.
3	0·1 c.c.	+ overnight.
4	0·1 c.c.	+ overnight.
5	0·05 c.c.	+ 36 hours.
6	0·05 c.c.	+ 36 hours.
7	0·025c.c.	+ 36 hours.
8	0·025c.c.	−
9	0·01 c.c.	−
10	0·01 c.c.	−

+ = Died. − = Lived.

During our work on lamb dysentery, we made an anti-toxin for *B. welchii*, and the occasion arose to test serum of normal horses for the presence of such anti-toxin in the hope of finding one with an appreciable quantity and which, therefore, could be immunised rapidly. The intradermic test was carried out on guinea-pigs, using a mixture of toxin and horse serum. The toxin produces a circular raised swelling which in time becomes necrotic and sloughs. A mixture of anti-toxin and toxin fails to produce such a lesion. Table IX. shows the results of intradermic injections of mixtures of a known toxin and normal horse sera.

TABLE IX.

Toxin.	Serum.	Result.
0·025c.c.	——	+
0·05 c.c.	——	+
0·025c.c.	0·1 c.c. H.1380	+
0·05 c.c.	0·1 c.c. H.1380	+
0·025c.c.	0·1 c.c. H.1440	+
0·05 c.c.	0·1 c.c. H.1440	+
0·025c.c.	0·1 c.c. H.1305	—
0·05 c.c.	0·1 c.c. H.1305	—
0·025c.c.	0·1 c.c. H.1413	—
0·05 c.c.	0·1 c.c. H.1413	—
0·025c.c.	0·1 c.c. H.1108	—
0·05 c.c.	0·1 c.c. H.1108	?
0·025c.c.	0·1 c.c. H.1471	—
0·05 c.c.	0·1 c.c. H.1471	—

From the six horses' serum tested, three proved to contain anti-toxin and a fourth may have had a trace. Horse H.1471 was further tested, this time using mice for the test. Table X. shows that 0·1 c.c. of the serum protected against 0·1 c.c. toxin—a certain M.L.D.

TABLE X.

Mouse.	Toxin.	Serum.	Result.
1	0·5 c.c.	——	Dead 2 hours.
2	0·5 c.c.	——	Dead 2 hours.
3	0·25 c.c.	——	Dead overnight.
4	0·25 c.c.	——	Dead overnight.
5	0·1 c.c.	——	Dead overnight.
6	0·1 c.c.	——	Dead overnight.
7	0·5 c.c.	0·25 c.c.	Dead overnight.
8	0·5 c.c.	0·25 c.c.	Dead overnight.
9	0·5 c.c.	0·1 c.c.	Dead overnight.
10	0·5 c.c.	0·1 c.c.	Dead overnight.
11	0·25 c.c.	0·25 c.c.	Dead overnight.
12	0·25 c.c.	0·25 c.c.	Dead overnight.
13	0·25 c.c.	0·1 c.c.	Dead overnight.
14	0·25 c.c.	0·1 c.c.	Dead overnight.
15	0·1 c.c.	0·25 c.c.	—
16	0·1 c.c.	0·25 c.c.	—
17	0·1 c.c.	0·1 c.c.	—
18	0·1 c.c.	0·1 c.c.	—

In the production of the anti-toxin for use on lambs, Horse H.1471 was used, and readily produced an

anti-toxin of high protective value, 0·005 c.c. protecting against 0.5 c.c. toxin, i.e., it had increased 100 times. Table XI. shows the titration at this period.

TABLE XI.

Mouse.	Toxin.	Serum.	Result.
1	0·5 c.c.	0·1 c.c.	—
2	,,	0·1 c.c.	—
3	,,	0·05 c.c.	—
4	,,	0·05 c.c.	—
5	,,	0·025 c.c.	—
6	,,	0·025 c.c.	—
7	,,	0·01 c.c.	—
8	,,	0·01 c.c.	—
9	,,	0·005 c.c.	—
10	,,	0·005 c.c.	—
11	,,	0·0025c.c.	Dead overnight.
12	,,	0·0025c.c.	Dead overnight.
13	,,	0·001 c.c.	Dead overnight.
14	,,	0·001 c.c.	Dead overnight.

Mr. Gaiger has told us that *B. welchii* is found in association with lamb disease, but I feel that he does not attach the importance to it that it warrants. I may say here that I have failed to isolate the *Vibrion septique* from any organ of the body in lamb disease, and other than present in the intestine in such cases I have not met it. It may be that I have not done a sufficient number of cases, but in practically every case I have examined I have been able to identify *B. welchii*, usually associated with *B. coli*, and sometimes with a diplococcus. I have, however, this season been able to examine under satisfactory conditions six lambs in various stages of the disease. Four lambs were dealt with in a veterinary surgeon's surgery, and two were able to be taken alive from the North of England to our laboratory in the South, and there killed in the laboratory. Specimens were taken with full aseptic precautions.

It will make an interesting study to observe the effect of feeding healthy lambs on cultures of *Vibrion septique* or mixtures. During the past season I was privileged to conduct some feeding experiments on a farm where the disease is unknown. Prior to carrying out these feeding tests, some were done at the labora-

tory. Table XII. gives the results showing that it required a mixture of *B. coli* and *B. welchii* to reproduce the disease.

TABLE XII.

Lamb.	*B.coli.*	*B.welchii.*	Result.
A	50 c.c.	——	–
B	——	50 c.c.	–
C	20 c.c.	20 c.c.	Dead 48 hours.
D	10 c.c.	10 c.c.	Dead 48 hours.
E	50 c.c.	50 c.c.	Dead overnight.
F	20 c.c.	10 c.c.	Dead 3 days.

The reproduction of the disease in the field could not be brought about by similar feeding so long as one left the lamb with its mother. Removal from its mother, and feeding on cow milk or withholding all milk had the desired result. Table XIII. will show these results.

TABLE XIII.

Group A (Lambs outside) fed on :—

(1)	Normal ewe's milk ...	No result.
(2)	Cow's milk	No result.
(3)	Infective ewe's milk ...	No result.
(4)	All milk withheld ...	Died of lamb disease.

Group B (Lambs kept inside building) fed on :—

(1)	Normal ewe's milk ...	No result.
(2)	Cow's milk	Died of lamb disease.
(3)	Infective ewe's milk ...	Died of lamb disease.

A very big question is opened up as to the part played by the ewe and her milk in the production or of protection from the disease—a question that will have to wait till next season for further study.

Mr. Gaiger says "that *B. welchii* is not of primary etiological importance in this disease is indicated by the fact that an anti-serum for this organism does not seem to have an influence in stopping the onset of a natural attack." I do not for a moment suggest that every case of lamb dysentery will be prevented

by the use of a *B. welchii* antitoxin, nor have I many figures to bring forward to say it will lessen the occurrence of the disease. I also know that in some districts where such a serum was used there was little differrence in the results between the inoculated and control uninoculated death rate, but on one farm in Scotland where 390 lambs were inoculated thirty died of lamb dysentery, while of the seventy-five lambs kept as controls under exactly similar conditions, and at the same time, thirty also died, giving death rates of 10 per cent. and 40 per cent. respectively. The inoculated lambs here had one inoculation only of a pure welchii antitoxin. The farmer says "the uninoculated lambs mostly died at two or three days' old, or, anyhow, at less than a week; no inoculated lambs died before nine days from the date of their inoculation."

Further tests were carried out last year using a mixture of *B. welchii* antitoxin and *B. coli* antiserum on lambs soon after birth, and repeating it each week for three weeks. On one farm thirty lambs were done, and put into a reputedly bad field—two only died of the disease, while fourteen of the thirty control lambs developed lamb dysentery and died. I give the following experience for what it is worth. On an infected farm seven ewes only had to lamb, and as one knows the last ewes to lamb lose almost all their lambs. Six were inoculated with the double serum, but from pressure of work the last to be born was left undone. It was the only one of the seven that developed the disease and died. I still have hope of preparing material from *B. welchii* alone or from *B. welchii* and *B. coli* for use either on the ewe before lambing, or on the lamb soon after birth, to produce some immunity to this scourge.

Some General Debatable Points.

(*a*) *Media for the Cultivation of Anærobes.*

I agree with Mr. Gaiger that a medium containing liver extract is excellent for the growth of all anærobes, but I do not agree that even when freshly made it will start a culture of *B. chauvœi* growing. If *B. chauvœi* has been kept in culture for some months without subculturing, a liver broth medium will not always produce a culture when inoculated. The

medium giving best results in our experience is Tarozzi's, a meat broth containing 1 per cent. glucose, to which has been added a small piece of uncooked sterile guinea-pig liver. The results are most gratifying.

(*b*) *Virulence of B. chauvœi.*

Mr. Gaiger states that *B. chauvœi* retains its virulence for years in artificial culture. I cannot agree with this statement, as our experience is exactly the reverse. Much time has been lost in some of our experimental work, because of the rapidity with which our cultures of *B. chauvœi* have lost their virulence. As an example of this let me quote the following :—

A meat broth culture of *B.chauvoei* killed guinea-pigs on intramuscular inoculation in doses of 0·3 c.c. in 24—48 hours. It had been left untouched in the same culture tube for six weeks, and on retesting for virulence was found to produce death only in doses of 3 c.c. By repeated passage it has been found possible to obtain a culture having the original lethal dose for guinea-pigs. Personally, I would feel very grateful if someone could give us some enlightenment on this point.

(*c*) *Suprarenal Reaction in Vibrion septique Infection.*

One reads commonly in text-books and articles describing guinea-pig infection with cultures of *Vibrion septique* that the suprarenals are hæmorrhagic. The veterinary department of our laboratory have *post-mortem* examinations on such guinea-pigs practically every day, and I cannot call to mind this feature. Possibly it only occurs in delayed deaths or in early deaths, but our experience does not show it to occur in animals which die overnight.

(*d*) *Filaments on Peritoneal Surface of the Liver.*

While I agree that the presence of filaments on the peritoneal surface of an injected guinea-pig's liver is of considerable service in the identification of *Vibrion septique,* I cannot confirm that it is absolutely diagnostic. While working with some commercial blackleg products from an antigenetic point of view many guinea-pigs had to be inoculated with the various samples. Some guinea-pigs died as a result of the inoculation, and in the case of one product filaments were present on all the dead guinea-pig

livers. The organism isolated from these dead guinea-pigs is certainly not *Vibrion septique*, though giving the liver surface reaction.

(*e*) *'Reading' Bacillus.*

It was quite a pleasure to me to see a reference in the paper to the 'Reading' bacillus, as I feel to some extent responsible for its introduction to the veterinary profession, having used it and advocated its use during the war days in France. I am rather surprised that its use has not been more generally adopted in the treatment of wounds. Certainly it is not a pleasant dressing to use, but the satisfactory results accruing from its application in suitable cases amply justifies its use, and compensates for any disagreeable appearance or odour during the period of treatment. Given the suitable case—a deep discharging wound with unhealthy or dead tissue to be sloughed out—an application of the Reading bacillus culture will surely effect a change in the course of a day or two. Naturally, where dead bone, etc., is present it can do little, and I feel that failures recorded may be due in a large measure to its misuse.

(*f*) *Solid Paraffin in Liquid Media.*

Personally I do not adopt the solid paraffin method for the exclusion of air from liquid media. There always exists the risk of its shrinking from the edges of the culture tube, and therefore undoing its usefulness. There is also the difficulty of withdrawing any fluid for examination: the paraffin has to be melted and a pipette used. With liquid paraffin things are more satisfactory, as it is not at all essential to make use of a pipette for the withdrawal of fluid, for by tilting the tube the paraffin can usually be made to keep to the surface when the liquid exudes from below. But in the majority of anærobic mediums no surface seal seems necessary at all: this is especially so with a liver medium or one containing cooked or uncooked tissue.

(*g*) *Mc Intosh and Fildes' Jar.*

Mr. Gaiger describes a very elaborate sample of the McIntosh and Filde's jar used in the cultivation of anærobes. Certainly if one has such a jar, by all means use it, but to my mind some of the simpler forms are quite satisfactory. Nor do I think it necessary to evacuate the air with a vacuum pump

before passing in the hydrogen. For the growth of anærobic colonies on the surface of solid media, the McIntosh and Filde's method has been advocated, using a capsule of palladiumised asbestos immediately within the rubber stopper at the mouth of a wide-necked bottle containing the medium. To my mind such a method is not essential—quite good results with many anærobes can be got by the use of a direct strong stream of sterile hydrogen for the displacement of the air.

(*h*) *Keeping Animals at Room Temperature before making a post-mortem.*

Referring to the recovery of *B. chauvœi* from inoculated guinea-pigs, Mr. Gaiger says: "If death has been rapid, cultures from the heart blood will be sterile, but if the dead guinea-pig is kept a few hours at room temperature, positive pure cultures may be obtained." Personally I regard the delay of the conducting of a *post-mortem* for bacteriological work as full of considerable risk because of the invasion of other organisms. This has been brought home to me very forcibly because of a little incident that occurred lately. Starting with a pure culture of *B. chauvœi*, one that has been used and tested for purity many times, I attempted to raise its virulence by direct passage of guinea-pig muscle juice. Having used four animals in this way, taking the greatest precautions possible, a fifth died from a very small dose. On bacteriological examination of this subject, a mixture of organisms was obtained from the heart blood, including *Vibrion septique*. I account for this result by having left the fourth animal too long at room temperature before making the *post-mortem*.

(*i*) *Bacillus Botulinus.*

I should like to draw attention to one or two points under this heading contained in the paper. Mr. Gaiger quotes that Madsen's filtered toxin was fatal to guinea-pigs in a dose of 0·0015 c.c. An American strain of *B. botulinus* used in our laboratory produces a toxin when filtered that is fatal to guinea-pigs in a dose of 0·00001 c.c., which when given intraperitoneally produces death in 3—4 days. Larger doses prove fatal earlier.

It is also stated that "the toxin of *B. botulinus* is relatively stable." Toxins prepared by us have not

this feature : there is a marked falling off in potency after keeping for seven days even in an ice room.

Cultures of *B. botulinus* have been injected subcutaneously and intraperitoneally into guinea-pigs by us with a view to its recovery from the tissues. We have never been able to obtain cultures from the heart blood, but have repeatedly been successful in growing cultures from the liver where actual pieces of the organ were inserted into the culture tube.

Feeding guinea-pigs by mouth with whole culture of *B. botulinus* has, in doses of 1 c.c., produced death in times varying from 18 hours to two days. On no occasion have we been able to recover the organism from any tissue of the dead fed animal.

I do not want to enter into a discussion on grass disease, and the relation between it and *B. botulinus*, but before I conclude I must make reference to two points.

Dr. Tocher's Organism.

It was only very recently that I knew there existed in our laboratories a culture of the organism isolated from the spleen of a case of grass disease by Dr. Tocher. I took the first opportunity of using it to feed guinea-pigs with. Four animals were fed with 1 c.c. of a 48 hours' culture—a pipette being used for the feeding. All four animals died in from 18—48 hours. If, as we are forced to believe, the only true diagnostic test for *B.botulinus* is that it shall be pathgenic by feeding, then the culture I used contains *B. botulinus*. I have had no time to do anything further with the culture yet. I have had the good fortune to be able to conduct some four *post-mortems* on grass disease cases, but never have I been able to isolate an organism in any way similar to *B.botulinus*.

B. botulinus Antitoxin in the Blood of Affected.

During the past few months I have been able to obtain samples of blood from three cases of grass disease. One was from a recovered case, and two from sub-acute cases which had been affected some six weeks.

The serum on being used in large doses failed to protect guinea-pigs against one M.L.D. of *B. botulinus* toxin. There was, therefore, no detectable antitoxin present.

With much of the experimental work on anærobes I have recorded, my colleague, Mr. H. R. Allen, M.R.C.V.S., has been associated.

In conclusion, let me once more say how deeply indebted I am personally to Mr. Gaiger for bringing forward this subject, for the manner in which he has dealt with it, and for the opportunity he has given me to express some opinions on anærobic infections of animals, and to place some of the results I have obtained before you.

Mr. Tudor Hughes: After the exhaustive and masterly opening to which we have just listened from Mr. Dalling, I do not desire to detain you, but I should just like to thank Professor Gaiger for the trouble he has taken in preparing the slides, and to make one suggestion, in this regard—as to the possible value of showing a section of the structure of the normal before showing the section of the abnormal. I was struck, on reading the paper, with the amount of gas evolved by these anærobes (loud laughter, in which Messrs. Dalling and Hughes heartily joined)—and whilst we are very interested as to what these organisms will grow in, and the characters they evince, as clinicians and practitioners we should like to know what they will not grow in; that is to say, what is the minimum concentration of the most suitable antiseptic to inhibit their growth. I think they account for the group of the most hopeless cases which we are called upon to treat. Concerning experimental inoculation, we have heard of a good many intravenous inoculations, and the question is, whether in experimental work the conditions of artificial inoculation should not conform to the conditions which prevail in the field. These anærobes are associated with conditions of rapid putrefaction, and there is thus a question whether their recognition would be of any value in forming a conclusion concerning the carcasses of those animals which are supposed to have died from lightning stroke.

Professor H. A. Woodruff: I want first of all to tender my congratulations to Professor Gaiger, not only for the excellence of his paper, but for the very high standard of work which lies behind the paper. I want to say one or two words about braxy. My predecessor in Melbourne, Professor Gilruth, did a

lot of work on this disease in three different parts of Australasia—first in New Zealand, then in Tasmania, and lastly in Victoria. The point which I want to make is that the pathological factor of frost and cold has nothing to do with the production of the disease, as we see it in various conditions (those prevailing in the different districts in which Professor Gilruth worked) in Australia. In different parts of the world you may find one and the same disease arising with pathological secondary factors which give you very great differences both in symptomatology and *post-mortem* appearance. This is one of the reasons why the hæmorrhagic lesion in the fourth stomach is not, with us, so important a factor as it is with you. Since a secondary factor with us is drought, our braxy occurs at a different time. We have been hanging fire in my own laboratory for some four years, hoping to have an outbreak—I speak from the point of view of the pathologist (laughter)—to furnish the material, and so far we are still waiting, but I am quite sure that when the opportunity does present itself, we shall apply Professor Gaiger's finding with very great interest. I wish to emphasise the importance in all our work upon the etiology of diseases of the class which we are now discussing, of securing our material, whenever possible, from the live subject, and, when this is not possible, from subjects which have had *post-mortem* examinations made upon them without loss of time.

It has been my privilege recently to spend some time in Jensen's laboratory, and in the High School at Copenhagen, and the organism with which they are working is this same organism with which Professor Gaiger is working.

With regard to the subject of *B. botulinus* infection, we find the disease in Australia in particular where there is a soil deficiency, especially in lime and phosphates, and we met with a disease which was often killing large numbers of cattle, and associated with this disease we found a habit of bone chewing to a peculiar degree. The cattle appear to absorb a toxic substance with the material ingested on sucking the bone, and we noticed a paralysis of deglutition. We have found that in the districts where this disease was prevalent, with the use of lime and superphos-

phates on the land it has become less and less frequent. I pay a tribute to my colleague, Dr. Seddon, for the work he has done on this subject. (Applause.)

Professor Gaiger's Reply.

Professor Gaiger, in reply, said: I do not want it to go out from this meeting that I in my way advocate *post-mortem* examination on dead animals. For years it has been a primary point that I have always insisted upon that you must get the animal in the live state, and get it some considerable time before you expect it to die. With regard to Mr. Dalling's points, I do not think there is anything upon which we disagree very seriously. I am extremely glad that Mr. Dalling has opened the discussion, because we have had the advantage of two papers instead of one. This point of the *Bacillus chauvœi* not retaining its virulence might be due to his media becoming too acid for his organism to survive for any length of time. He will have better results if he dries the muscle, and preserves the dried muscle. When I said that the botulinus toxin was stable, I meant that it was relatively stable compared with the unstable toxins formed by such organisms as the *Vibrion septique* and *B. welchii.*

It is news to me that the organism isolated by Dr. Tocher and kept at the Wellcome Research Laboratories, is killed by feeding. I cannot help thinking that there must be some mistake about this, because I think this is a claim which Dr. Tocher himself has never made for his organism. It is easy to get a tube wrongly labelled. (Laughter.) I quite agree with what was said about the necessity for laboratory experimentation being confirmed by field experiments. What we are waiting for in regard to braxy is an extremely bad year, so as to see what effect the vaccine against braxy has upon such conditions. It is very interesting to hear from Professor Woodruff that frost has no effect in New Zealand. The veterinary surgeon generally lays too much stress upon frost—the point is that anything that lowers the tone of the stomach may produce a case of braxy, if bacterial infection takes place. Professor Woodruff also spoke about the periodicity of the disease. We know that in this country that

also occurs. The mortality from braxy is almost invariably rated too high by farmers. When you have actual observation you do not get a death rate in braxy of 30, 40, or 50 per cent.

There is no bone-eating habit in grass disease in horses. I can quite understand that, where the craving occurs for phosphorus, animals will eat bones, and in that way the toxigenic bacillus gains entrance, but these horses do not have the opportunity of getting bones.

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